Project number: 5R01HD056999

Title: Vitamin D: a Link to Racial Disparities in Adverse Birth Outcomes

Contact PI: Bodnar, Lisa M.

Awardee organization: University of Pittsburgh

Main analytes: 25-hydroxyvitamin D, high-sensitivity C-reactive protein, genotyping

Description of the project: This proposal will address gaps in our understanding of the intractable racial disparities in preterm birth and preeclampsia by exploring novel mechanisms underlying these pathways. We focus on maternal vitamin D, a previously unexplored candidate influence on pregnancy outcome. We have shown that vitamin D deficiency is pervasive among black women throughout pregnancy and is significantly less common among white women. Further, new data indicate that vitamin D has direct and indirect influences on inflammation and other known molecular pathways in the pathogenesis of spontaneous preterm birth (sPTB) and preeclampsia. Therefore, vitamin D status is an unexplored risk factor for sPTB and preeclampsia. The goal of this reproductive epidemiologic study is to elucidate the interplay between maternal vitamin D status, inflammation, and polymorphisms in genes involved in vitamin D metabolism on the risk of sPTB and preeclampsia. We will conduct complementary analyses in two racially- and geographically diverse multi-center U.S. prospective cohorts: the **Collaborative Perinatal Project** (n=55,908; 46% white; 47% black) and the NICHD Maternal-Fetal Medicine Units Network High-Risk Aspirin Study (n=917 pregnancies; 33% white, 57% black).

First, we aim to determine the independent association between maternal vitamin D status and the risk of sPTB and preeclampsia, and identify the extent to which this association is modified by maternal race. Vitamin D status at ≤26 weeks' gestation will be assessed using serum 25-hydroxyvitamin D. Second, we will evaluate the independent effect of maternal vitamin D status on maternal inflammation, and identify the extent to which this association is modified by maternal race. Maternal inflammation will be assessed using high-sensitivity C-reactive protein (CRP) measured at ≤26 weeks' gestation. Finally, we will determine the effect of maternal and fetal/neonatal genetic variation in key vitamin D metabolic loci on maternal vitamin D status, and on the risk of sPTB and preeclampsia. We will study genes whose protein products are directly involved in the metabolism of vitamin D: 25-hydroxylase (*CYP27A1*), 1a-hydroxylase (*CYP27B1*), vitamin D binding protein (*GC*), 24-hydroxylase (*CYP24A1*),

vitamin D receptor (*VDR*), and retinoic acid receptor alpha (*RARA*). This project is highly responsive to the NIH Roadmap Initiative, given the confluence of expertise across epidemiologic, clinical, and biological sciences. Moreover, the project is significant because maternal vitamin D status is modifiable. Interventions to improve vitamin D status, such as a moderate increase in sunlight exposure or vitamin D supplementation, are inexpensive, safe, and acceptable to women. Given the high proportion of vitamin D inadequacy among black women, and the profound impact sPTB and preeclampsia have on perinatal morbidity, this project has tremendous capacity to benefit public health.

Specific aims:

Specific aim 1: To determine the independent association between maternal vitamin D status and the risk of spontaneous preterm birth (sPTB) and preeclampsia and to identify the extent to which this association is modified by maternal race.

Specific aim 2: To evaluate the independent effect of maternal vitamin D status on maternal inflammation.

Specific aim 3: To determine the effect of maternal and fetal/neonatal genetic variation in key vitamin D metabolic loci on maternal vitamin D status, and on the risk of sPTB and preeclampsia.

Prioritized list of assays: We request maternal serum for 3 assays listed below. The total volume requested is 0.32 ml (320 µl), and it is prioritized as listed below. We request that, where sample volume allows, 3 aliquots are sent to us, so that we may send each aliquot to the external labs without additional thawing.

1) 25-hydroxyvitamin D. Serum 25(OH)D is the clinical indicator of vitamin D nutritional status [1,2]. Serum will be sent to Dr. Michael Holick at Boston University for analysis of total 25(OH)D ($25(OH)D_2$ + $25(OH)D_3$) using liquid-chromatography-tandem mass spectrometry [3]. Serum samples will be prepared and analyzed through a turbulent flow LC system followed by traditional laminar flow chromatography and are then be analyzed relative to the control solutions for detection and quantification of the 25(OH)D. The analysis will be performed using a TSQ Quantum Ultra triple mass-spectrometer (Thermo Finnigan Corp., San Jose, CA). The intra-assay coefficient of variation is 6.0%. ***0.12 ml (120 µg) of serum is required for this assay***.

2) High-sensitivity C-reactive protein. CRP is a reliable index of chronic systemic inflammation [4]. Serum high-sensitivity CRP will be measured in the laboratory of Dr. Simhan at the Magee-Womens Research Institute using ELISA kits. The detection limit of the CRP assay is 0.2 ng/ml, with intra- and inter-assay variabilities of 4% and 7%, respectively. ***0.02 ml (20 µg) of serum is required for this assay.***

3) Genotyping. We will study genes whose protein products are directly involved in the metabolism of vitamin D including: 25-hydroxylase (*CYP27A1*), 1a-hydroxylase (*CYP27B1*), vitamin D binding protein (*GC*), 24-hydroxylase (*CYP24A1*), vitamin D receptor (*VDR*), and retinoic acid receptor alpha (*RARA*). All methods will be carried out in the molecular epidemiology laboratory of Joseph Zmuda at the University of Pittsburgh Graduate School of Public Health. We will isolate high molecular weight genomic DNA from cells concentrated from serum aliquots using the QiaAMP DNA Kit (QIAGEN Inc., Valencia, CA). We will "bulk up" the DNA using the multiple displacement amplification method of whole genome amplification [5] with the REPLI-g kit (Qiagen) [6]. Amplified DNA will be quantified by the PicoGreen method. We will use the Sequenom MassARRAY iPLEX Gold platform as the primary method for genotyping of VDR SNPs. We have extensive experience using this platform and have found excellent call rates and reproducibility of genotype calls. The iPLEX Gold assay combines the benefits of robust single-base primer extension biochemistry with the sensitivity and accuracy of MALDI-TOF mass spectrometry detection. The iPLEX® Gold assay is based on multiplex PCR followed by a single base primer extension reaction. After PCR, remaining nucleotides are deactivated by SAP treatment. A single base primer extension step is performed, and the primer extension products are analyzed using MALDI-TOF mass spectrometry and cluster analysis and genotype calling are completed using Typer Software. ***0.2 ml (200 µg) of serum is required for these assays***.

References

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3. Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, Petruschke RA, Chen E, de Papp AE. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005; 90(6): 3215-24.
4. Kluft C, de Maat MP. Sensitive markers of inflammation make it possible to study the chronic process: the rise of interest in low levels of C-reactive protein. Vascul Pharmacol 2002; 39(3): 99-104.
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6. Hosono S, Faruqi AF, Dean FB, Du Y, Sun Z, Wu X, Du J, Kingsmore SF, Egholm M, Lasken RS. Unbiased whole-genome amplification directly from clinical samples. Genome Res 2003; 13(5): 954-64.